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BIOCHEMICAL PROFILING OF BAMBOO SEEDS (*MALOCANNA BACCIFERA*) AND EFFECT ON REPRODUCTION AND CHROMOSOMAL ABNORMALITIES IN THE FEMALE RAT (*RATTUS NORVEGICUS*)

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ABSTRACT

Experiments were performed on seeds of bamboo (*Malocanna baccifera*) and female rat (*Rattus norvegicus*) to see the biochemical profile and its effect on the reproductive potentials respectively. The bamboo seeds that are collected after a sporadic flowering were examine for present of steroid, total phenol, total flavonoid and antioxidant properties. Bamboo seeds shows the presence of steroid in both the epicarp/ mesocarp and endosperm. The total phenolic contain showed higher concentration in the extract of epicarp/ mesocarps while total flavonoid content did not very. The lower LC_{50} of epicarp/ mesocarps when compared to endosperm indicates batter antioxidant properties in bamboo seeds. The estradiol concentration in the female rat feed with the bamboo seeds did not differ significantly with that of the control group. The cytological observation shows mostly normal distribution of chromosome numbers 2n=42 in most of the plate but some the numerical abnormalities in chromosome numbers with 2n=45, 44, 43, 38, 30 were also observed in some of the plates. Robertsonian fusions and B- chromosomes were also reported in the present study. Thus it would be too early to conclude and link the bamboo flowering and increase in rat population.

KEYWORDS: Antioxidant, Bamboo, Manipur, Phenol, Steroid

INTRODUCTION

Manipur is situated in the North - Eastern corner of India, bordering with Myanmar. It has a rich flora and fauna, and is one among the biodiversity hotspots of the world (Singh *et al.* 2009). According to the Statistical Bulletin of Manipur Forests (1999-2000), bamboo forest cover about 21.57 % of the total forest area. There are many incidences of gregarious and sporadic bamboo flowering in North - East India (Dhananjoy *et al.* 2012; Rathaur 2013). It is generally believe that there exists a correlation between bamboo flowering and explosion of rat population particularly in North - East India (John and Nadgauda 2002) including Manipur. The bamboo seed are intermittent in nature for rats and is available in plenty to feed on only during the flowering periods. As the seeds started germinating, rats are shortage of foods. This suddenly forced them to invade nearby farm and cause havoc with the standing crop (John and Nadgauda 2002). It even invade the granary of the nearby villages. Thus, leading to believe that bamboo flowering is the indication of upcoming famine to the people of North - East India (Nag 2001) and to the world (Unwin 1927). Bamboo is an inseparable part in the life of the people of Manipur as most of the species of bamboo are utilized in different domestic and commercial purposes. Further, there is reports of seeds being used as staple food grain (Karuba *et al.* 2007). Bamboo seeds are very

reach in nutrients. The protein content of the seeds are comparable with that of wheat but superior to rice thus excelling both rice and wheat in nutrient value (Karuba *et al.* 2007). The seeds also contained all the essential amino acids (Lakshminarayana *et al.* 1955).

Voluminous literatures exist on the bamboo flowering and its relation to the increase in population of rat, mostly based on the survey works (Nag 2001; John and Nadgauda 2002; Pathak and Kumar 2004). While the experimental observation on the biochemical aspects of bamboo seeds/fruits and its effect on the rats are rarely examined. Thus, it will be a novel experiment to study the biochemical contains of bamboo seeds along with the observation of the reproductive potential and chromosomal abnormalities in the female rats as it is the sole responsible for the increased in rat population. Therefore, the present study was conducted on the seeds of bamboo, *Melaconna baccifera* to see the biochemical profiling of some of the chemicals i.e., present of phenol, steroid, flavonoid and the antioxidant property in the seed extract. Further, the observation on the blood serum levels of gonadal steroid (estradiol-17β) and chromosomal abnormalities of the female rats (*Rattus norvegicus*) that feeds on the bamboo seeds will give a brief knowledge on the reproductive potential in these rat. Further, the present study will give some scientific inputs to the present date available in to the myth of bamboo flowering and harbinger of famine.

MATERIALS AND METHODS

254 bamboo seeds of *M. baccifera* were collected from Kwatha (Moreh, Manipur) during the month of April, 2012 where sporadic flowering of bamboo plants occurred. They were cut into small slices so that the epicarp/ mesocarps and endosperm were separated and shed dried separately. These two parts were separately extracted using methanol. To examine the effect of bamboo seed on reproductive and chromosomal abnormalities of female rat, 11 numbers of albino female rats (*R. norvegicus*) were captured from the wild during the same period from nearby area where bamboo flowering take place. They were brought to the animal house and acclimatized for 5 days under captivity. These rats were divided in two group, one group (n=6) was feed only with the fresh bamboo seeds (cut pieces of both epicarp/mesocarp and endosperm) and water *ad labitum* and the other group (n=5) was kept as control (normal food consisting of paddy seeds, potato and milk powder) for 2 weeks. Regular virginal smear examination was carried out regularly to see the estrus cycle of the female rat and counted as day 0. The samples were collected before the ovulation.

Present of Steroids in the Extracts

The extraction of steroids from the bamboo seeds were carried out as mention in the Harborne (1973). The present and absent of the steroids in the extracts were confirmed by performing the following examinations:

- Red colour produced indicates the presence of steroids in the lower chloroform layer when 2 ml of the extract was dissolved in 2 ml of chloroform and 2 ml of concentrated sulphuric acid.
- The development of a greenish colour indicates the presence of steroids when 2 ml of the extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acids.

Total Phenolic Content

The total phenolic content (TPC) was determined by the Folin–Ciocalteu colorimetric method (Singleton and Rossi 1965). The absorbance was determined at 725nm with a UV-Visible spectrophotometer (Multiskan spectrum, Thermo Scientific) and compared with a gallic acid calibration curve. The phenol content was expressed as mg gallic acid

equivalents (GAE)/100 g of the extract.

Total Flavonoid Content

Total flavonoid content was measured by the Zhishen et al (1999) method of the $AlCl_3$ colorimetric assay. An aliquot (1ml) of extracts (V/W) or standard solution Catechin (Sigma –Aldrich) was added to 10 ml volumetric flask containing 4ml of distilled water. To the flask was added 0.3ml of 5% $NaNO_2$. After 5min, 0.3ml 10% $AlCl_3$ was added. Again 2ml 1M NaOH was added and the total volume was made upto 10ml with distilled water and the solutions were mixed well and the absorbance was taken against the prepared reagent blank at 510nm UV-visible (Multiskan spectrum, Thermo Scientific). Total flavonoid content of the extract was expressed as mg Catechin equivalents (CE)/ 100g dried mass.

Antioxidant Activity Assay

The DPPH free radical scavenging was assessed on each of the plant extracts (epicarp/ mesocarps and endosperm) with minor modification (Okada and Okada 1998). 0.1 mM DPPH radical solution in ethanol was prepared. Of 1ml different concentrations of extracts were prepared and the volumes were made uniformly to 1000µl. 5 ml of the DPPH solution was added. Ascorbic acid was used as a positive control at 1 mg/ ml. The mixtures were left standing at room temperature in dark for 30 minutes. After 30 minutes, the absorbance was measured at 517 nm on UV-visible (Multiskan spectrum, Thermo Scientific). The anti-oxidant activity was replicated three times per treatment. Decreasing the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. The radical scavenging activity, expressed as percentage of inhibition was calculated using the equation

Inhibition Concentration % = [(A-B)/A] x 100

Where A is absorbance of control (DPPH solution without the sample), B is the absorbance of DPPH solution in the presence of the sample (extract). IC_{50} value is the concentration of the sample required to scavenge the 50% DPPH free radical.

Estradiol Assay

Blood samples were collected from the rat before ovulation for the estimation of estradiol 17-β by using a commercially available ELISA kit (DiaMetra, Italy; product code: REF DKO003) following the protocol as mention in the kit. This kit has been validated and successfully tested in the estimation of estradiol in rat (Cos *et al.* 2006). Briefly, the blood samples were kept at room temperature for one hour and then centrifuged at 1500 rpm for 15min to collect the serum. The steroid were extracted from the blood serum by using diethyl ether (Singh 1985). This allow us to store the sample for longer period of time under -20^{o}C. The steroid extracted were reconstituted in the phosphate buffer before processing for ELISA. The samples were run in triplicate. The automatic microplate reader (BioRad iMark^{TM} Microplate reader) record the absorbance at 450 nm. The concentration of the estradiol was expressed as pg/ ml and can be distinguished from zero standard is 10 pg/ ml at 95 % confidence limit.

Chromosome Abnormalities Study

Study on chromosomal abnormalities was carried out by harvesting the bone marrow cells and the metaphases plates were obtained through flame dry method. It was then rinsed in water, dried and stained in 4% Sodium phosphate buffered Giemsa stain at pH 6.8 for 20 minutes. Permanent slide were then prepared by mounting them in DPX. At least

100 metaphase plates were counted and well spread plates were taken for the observation of aberration in Olympus BX 40 phase contrast microscope and snaps were taken with the digital camera attached to it.

Statistical Analysis

The data were presented as mean ± SEM and were analyzed using student's-t test to compare parameters between the two means of epicarp/ mesocarp and endosperm in bamboo seeds and control (normal food) and treated (feed on bamboo seed) in the female rat. All the experiments were conducted as per the guideline of the institutional ethical committee.

RESULTS

Results are presented in the figures 1 to 5. Both the steroid detection methods showed red and greenish color respectively with the extracts from endosperm and epicarp/ mesocarps of bamboo seeds indicating the presence of steroids in methanol extracts in all parts of fruits. This presence of steroids in all the parts of fruit lead us to further investigate the other biochemical parameters. Significantly ($P < 0.001$, student's - t test) higher concentration of total phenolic content was observed in the extract of epicarp/ mesocarps (5.55 ± 0.10 mg/100 g) when compared with the endosperm (1.23 ± 0.21 mg/ 100 g) in the bamboo seeds. The concentration of phenol in the epicarp/ mesocarps is about 4.5 times higher than the endosperm of the bamboo seed (Fig. 1). However, the total flavonoid content (epicarp/ mesocarp: 3.34 ± 0.12 mg/ 100 g and endosperm: 3.30 ± 0.24 mg/ 100 g) in both the extracts was found to be insignificant ($P = 0.2305$, student's t-test; Fig. 2) showing that the flavonoid are evenly distributed in all the parts of the seeds. Higher LC_{50} ($P < 0.001$, student's-t test) was recorded in the extract of endosperm (0.354 ± mg/ ml) than that of the epicarp/ mesocarp (0.159 ± mg/ ml). The lower LC_{50} value in epicarp/ mesocarp indicates better radical scavenging ability in the bamboo seeds. Thus, resultant in 2.2 times antioxidant activity more in the extract of epicarp/ mesocarp (Fig. 3). The study on the reproductive status of the female rat revealed that there is no significant changes ($P = 0.4870$, student's-t test) in the levels of estradiol-17β between the control (204.34 ± 20.4 pg/ ml) and those feed on bamboo seeds (207.63 ± 12.98 pg/ ml, Fig. 4). The control group of female rat, *R. norvegicus* has 2n = 42 chromosomes (Fig. 5E). Even after feeding it with the bamboo seeds, the chromosomal numbers was found to be normal i.e., 2n = 42 in most of the plates (14/ 100), however, in some of the plates numerical abnormalities was found to be composed of 2n = 45 (4 in number), 44 (14), 43 (12)(Fig. 5F), 39 (2), 38 (1), 41 (4), 40 (2), 34 (1), 32(2). The structural abnormalities, the Robertsonian fusions (4) (Fig. 5G) and the B-chromosomes that can divided as dot (7) and acrocentric (1) (Fig. 5 H and I respectively) and dot B chromosomes were predominantly present in most the chromosomal plates (Fig. 5 H).

DISCUSSIONS

The result obtained from the present study shows that the bamboo seed contain steroid in most parts of the seeds. This make us specious as this steroids might be helping the rat in increasing the reproductive potential during the bamboo flowering periods. There are reports that the shoots of the different species of bamboos contain a reach amount of phytosteroids and are used as a source by many pharmaceutical companies to extract active steroids from this plant (Srivastava 1990; Sarangthem and Singh 2003; Panday *et al.* 2012). Studies also reported that this bioactive phytosteroids help in reducing serum cholesterol level (Quilez *et al.* 2003) and also known to possess anticancer effects against lungs (Mendilaharsu *et al.* 1998), stomach (DeStefani *et al.* 2000), ovaries (McCann *et al.* 2003), and estrogen-dependent human breast cancer (Ju *et al.* 2004). The phytosterol have the inhibiting properties of on carcinogens, growth of cancer cells, cell

invasion, and metastasis (Giri and Janmejay 2000).

Phenolic compounds was found to contain more in the epicarp/ mesocarp when compared to endosperm of bamboo seeds in our study. However, the flavonoids were evenly distributed in the bamboo seeds. This compound are most commonly occurring groups of secondary metabolites and found widely distributed in the leaf, branch, trunk, root and fruit (Rispail *et al.* 2005; Nirmala *et al.* 2007). Further, the antioxidant properties was more in epicarp/mesocarp in our study seeds. Zhang *et al*. (2007) had utilized the leaves of *Phyllostachys nigra var.* as a source of phenolics and flavonoids and showed the antioxidant properties. Qinxue *et al*. (2012) reported the seasonal variation in phenolic, flavonoids and the antioxidant activity in the bamboo leaves of *Sasa argenteastriatus*. The phenolic, flavonoids and the antioxidant activity in bamboo leaf extracts was significantly higher in autumn and winter than in spring and summer (Qinxue *et al.* 2012). Normally in North-East India, peak flowering of the bamboo take place at the last month of the year and seeds mature at the beginning of the next year before the start of the rainy season (Rathur 2013). Thus providing large quantity of high quality food for the rat during this period. In a different study on the flavonoids accumulation in the Arabidopsis seed accessions by in vivo staining method with diphenyl boric acid 2-aminoethyl ester (DPBA), flavonoid were detected in both the embryo and endosperm in 6 h-imbibed col seeds suggesting that flavonoids accumulate at earlier stages, probably during seed development. Further, it was not detected in the seed coat (Endo *et al.* 2012). However, Lepiniec *et al* (2006) observed that flavonoids were found to accumulate in the immature rapeseed coats.

In the female rat, estradiol play roles in reproductive cycle and maintenance of pregnancy as well as the development of secondary sexual characters (Lubahn *et al*. 1993). This reproductive steroid hormone is commonly used in as a fertility detection by measuring the amount of circulating levels in the blood (Sjahfirdi *et al*. 2011). In the present study, the estradiol levels did not varies in the female rats of both the control and the group feeding on bamboo seed (Fig. 4), showing that there is no difference in the reproductive behavior. No doubt that both the groups of female rat are at the peak stage before ovulation. It was reported that reproductive health related problems were reduced by the regular intake of bamboo shoots (Nongdam and Tikendra 2014). However, in a different study by Yakubu and Bukoye (2009) confirmed that the leaves extract of *Bambusa vulgaris* have the abortifacient potentials in pregnant rabbits there by increasing the number of fetus dead as well as reduced survival ratio along with the decrease concentration on serum progesterone, LH and FSH. Similar was observation in male rat that reduced fertility when the ethanol extract of *Bambusa arundinacea* tander shoot was given (Rathaur 2013). Further, the phenolics compound also have bitter taste, offensive odor, toxicity or on slowing down digestion of food material and act mainly as protecting agents against mammalian herbivores (Freeland and Janzen 1940; Palo and Robbins 1991). It was further supported by a study on reproductive females of mountain hares (*Lepus timidus*) that the stomach contain plants with less phenolic contain (Iason and Waterman 1988) suggesting the possibility of deleterious effects on the reproduction and growth of mammals by the phenolic compounds (Dhananjoy *et al*. 2012).

CONCLUSIONS

The study on the chromosome number abnormalities on female rat showed 2n=45, 44, 43, 38, 30 in some metaphase plates along with Robertsonian fusions and B-chrmosomes while most of them were normal (2n=42). Yosida (1983) reported the spontaneous occurrence of polymorphism chromosome in nature in *Rattus rattus* chromosome no. 1, 9 and 13 which may be either by physical, chemical or radiations. In our previous study on wild rat during the bamboo flowering reported high polymorphism with a significant presence of sub telocentric in the chromosome pair s No. 1, 3, 5,

7, 9 and 13 suggesting abnormal chromosomal structure in rat by eating of bamboo seeds during the flowering periods (Dhananjoy *et al*. 2012). In a different study on the extract of fermented bamboo shoot, it was observed that the extract given to the mice produced some chromosomal aberration in somatic and germ cells (Guneshwor *et al*. 2007). Further, the effect of the extract was found to be dose dependent and possess genotoxic materials. The real causes of such abnormalities at chromosomal level is not known yet. Possibly, the steroid might cause it in higher concentration. An extensive study is needed in this aspect to come to a conclusive reasons about the myth of rodent population explosion and consequently resulting into famine and havoc due to bamboo flowering. We need a firm experimental results to prove/ disprove it.

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vitro and ovariectomized athymic mice. *J. Nutr.* **134** 1145-1151.

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APPENDICES

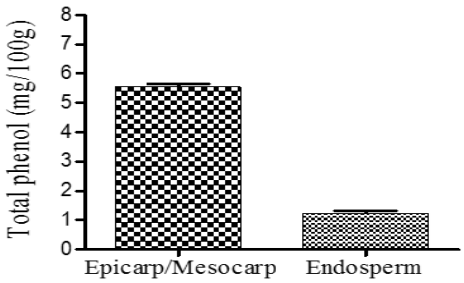

Figure 1: Total Phenolic Content in the Extracts of Epicarp/Mesocarp and Endosperm of *Melaconna baccifera*

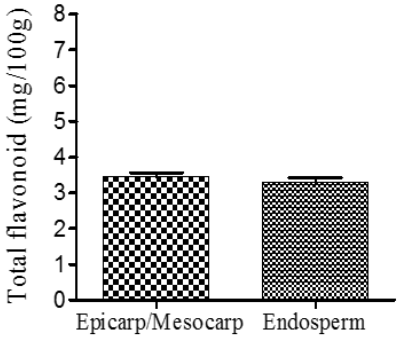

Figure 2: Total Flavonoid Content in the Extracts of Epicarp/Mesocarp and Endosperm of *Melaconna baccifera*

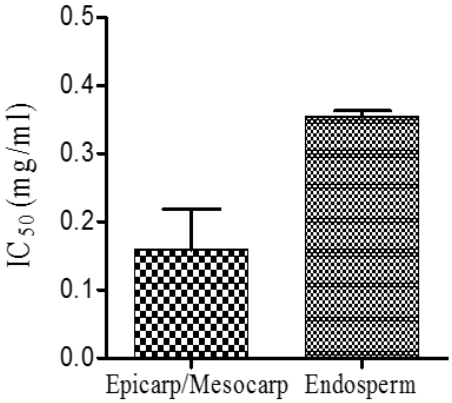

Figure 3: Antioxidant Properties in the Extracts of Epicarp/Mesocarp and Endosperm of *Melaconna baccifera*

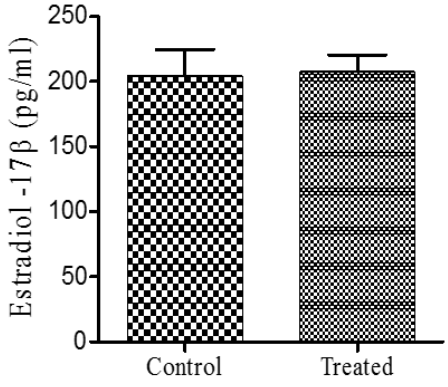

Figure 4: The Serum Concentration Levels of Estradiol-17 B in Female Rat Under Control (Normal Food) and Treated (Only Bamboo Seed Were Given as Food) Conditions

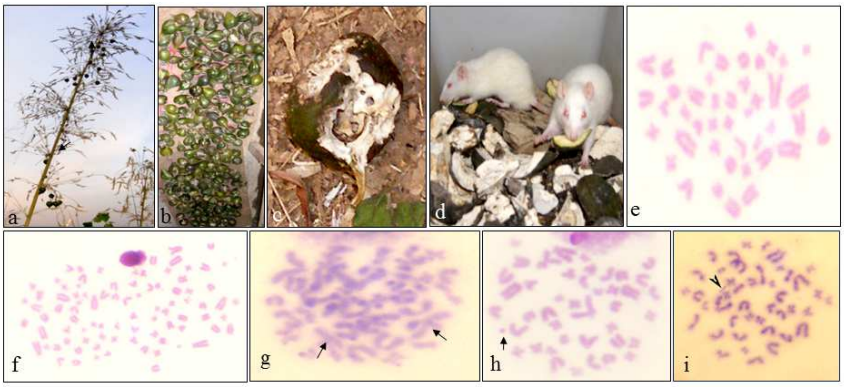

Figure 5: A: Bamboo Plant Bear Seeds after a Brief Period of the Sporadic Flowering in Kwatha, Manipur; B: Bamboo Seeds Collected from Various Places of Kwatha, Manipur; C: Bamboo Seeds Lying Half Eaten by the Wild Rat During the Bamboo Flowering Period; D: Albino Female Rats (*R. Norvegicus*) Feeding on Bamboo Seeds; E: Normal Chromosome Distribution in Albino Female Rats (*R. Norvegicus*, 2n=42) in Control Group; F: the Chromosomal Plates Indicating Numerical Abnormalities of 2n=42 (12); G: Arrow Indicating Robertsonian Fusions; H: Arrow Indicate B-Chromosomes Represented by a Dot and I: Arrow B-Chromosomes Presented by an Acrocentric